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MICROBIOLOGICAL CHARACTERISTICS OF THE SOIL AFTER CONVERSION OF TROPICAL FOREST AND PASTURE INTO CITRUS GROVE

Thais NUCCI[1] and Ely NAHAS[2*]
[1] *Program in Agropecuary Microbiology, Faculty of Agronomy and Veterinary Sciences/Universidade Estadual Paulista (UNESP), SP, Brazil*
[2] *Department of Crop Production, Faculty of Agronomy and Veterinary Sciences/UNESP, 14884-900 Jaboticabal, SP, Brazil*

Abstract

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Citriculture is expanding into forests and pastures. Such changes in land use provide an opportunity to evaluate differences in soil microbiological and chemical characteristics related to C, N, and P cycles between tropical forest, pasture, and orange grove (*Citrus sinensis* L. Osbeck). The aim was to identify soil characteristics related to the conversion of a sustainable forest system into pasture and later into orange crop. Soil samples were collected from areas of forest, pasture, and both the top (T/orange) and bottom (B/orange) of an orange grove. The numbers of bacteria and fungi, as well as potential nitrification, respiratory, ureolytic and phosphatase activities were determined. The lowest bacterial counts were found in the pasture soil and the highest in the forest soil. Fungi were significantly (p<0.05) more numerous in the orange grove soil than in the other soils. Respiratory activity in the forest and B/orange soils was lower than in the other soils. No difference was observed in the potential nitrification among the studied soils. Phosphatase and urease activities decreased in the following order: forest > pasture > B/orange > T/orange, although a significant difference was only found between the forest and T/orange soils. Significant differences in the organic matter content were found between the T/orange and the forest soils, and in the soil moisture between the T/orange and B/orange soils. In general, the microbiological and chemical characteristics found in the forest soil were less changed in the B/orange soil than in the T/orange and pasture soils. The characteristics studied related to the C, N, and P cycles were important to evaluate the impact of changes in soil quality.

Key words: citriculture, potential nitrification, respiratory activity, soil enzymes, soil quality

Introduction

There is increasing concern about losses of native forests for pastures and agriculture. About 21% of the original Brazilian forest has been deforested, including 7% in the Amazon, 9% Cerrado, 9% Atlantic Forest, and 5% Caatinga, Pampas and Pantanal. For example, from the 72 million hectares deforested in the Brazilian Amazon, 78% were converted to pastures (Braz et al., 2011). Brazil is the world's largest producer of oranges, producing 18.0 million tons corresponding to about 30% from total world production (Mapa, 2012).

The replacement of forests and pastures with citriculture may cause an imbalance in the ecosystem by changing soil properties. For example, cultivation of orange decreased soil pH, percent base saturation, organic matter, and the cation exchange capacity (Sanchez et al., 1999). Changes from forests to agriculture areas have been found to result in alteration of soil microbial processes (Chen et al., 2008). The conversion of forest into agricultural land may affect the organic matter content, microbial communities, and the processes of N mineralization and nitrification in the soil. During conversion of native forest into plantation forest, the mineralization rate, soil nitrification rate, and soil N concentration declined significantly (Yan et al., 2008).

Forest areas are subject to rapid degradation and deforestation, which decreases the C content (Ordóñez et al., 2008). Indeed, agricultural cultivation decreased the concentration of soil organic carbon, total N and P and the biological properties

* *Corresponding author:* enahas@fcav.unesp.br

of the soil such as basal respiration and enzyme activity by 18 to 38% (Su et al., 2004). In addition, the conversion of forest to corn or potato crops decreased the levels of total nitrogen, soil organic carbon, available phosphorus, and potassium (Liu et al., 2002). The microbial carbon and nitrogen biomass along with the urease and dehydrogenase activities decreased when a forest was converted to agriculture (Ralte et al., 2005). However, little is known about soils under citrus cultivation in areas that were previously under forest or pasture.

The microbial community of a soil plays a key role in the nutrient dynamics of different ecosystems, influencing the transformation of C, N, and P (Rezende et al., 2004). Therefore, microbial indicators have been considered useful for identifying changes in soil under different management systems. Soil characteristics such as enzymatic activity, respiration rate, and microbial biomass and diversity were used to monitor environmental change resulting from agricultural use (Allen and Schlesinger, 2004). Phosphatase activity was decreased when native forests were changed to monoculture larch plantations (Yang et al., 2010). According to Saviozzi et al. (2001), while the soil microbial biomass, respiration, and enzyme activities increased in pasture these attributes decreased in cultivated soil. Marinari et al. (2000) reported a significant effect on enzyme activity and CO_2 production on corn crop production due to the addition of organic and inorganic fertilizers that stimulated biological activity. However, Lucas et al. (2007) showed that although the addition of nitrogen sources can cause changes in microbial community structure, they do not necessarily have an impact on the activity of extra cellular enzymes. A considerable number of studies have been conducted to evaluate the effects of topography on biochemical parameters (Garcia et al., 2002; Sidari et al., 2008) but not on the quality and productivity of citrus grove.

Our objective was to study the microbiological characteristics of the soil after the conversion of a fragment of a tropical forest into pasture, part of which was later cultivated into an orange grove, in an area of sloping topography.

Materials and Methods

The study was conducted on Spring Farm, in Balbinos, São Paulo, Brazil (460 m altitude), in an area of 367.8 ha, composed of native tropical forest, pasture, and citrus (*Citrus sinensis* L. Osbeck), with a slope of 3%. Since 1987, part of the forest (346 ha) has been converted into pasture (*Brachiaria decumbens*) designed for cattle stocked at 1–2 animals per ha with pasture rotation. Since 1999, some of the pasture has been planted with orange (169 ha), with a spacing of 7 m between rows and 4 m between plants within rows, totaling 360 plants ha^{-1}.

The regional climate is subtropical and humid with a dry season from May to September, an annual rainfall of 1,700 mm and a temperature range of 19 to 30.4 ° C. The soil is a Dark Red Podzol according to Brazilian soil taxonomy (Embrapa, 2006) (Kanhapludult to US Soil Taxonomy), the chemical and mineralogical characteristics of which are presented in Table 1.

In 2003, lime and fertilizer were applied to the orange grove as follows: 7 Mg of lime ha^{-1} in July, 1.0 kg of superphosphate (18% of P_2O_5) in August, and 2.8 kg 20-5-20 formula (ammonium nitrate, superphosphate, potassium chloride) per plant split into three applications, the last being in March 2004. During 2004, lime and fertilizers were applied as in the previous year. The pasture and forest were not fertilized.

Soil samples were collected from the forest, pasture, and orange grove in March 2005. Soil samples were collected from the top (T/orange) and bottom (B/orange) of the orange grove. The altitude difference between the top and bottom of the orange grove was approximately 17 meters and the horizontal distance was 600 meters. In the pasture and forest, seven plots (seven replications) each measuring 100 m^2 was randomly selected. In each plot, three sub samples were randomly collected with the aid of a Dutch auger at a depth of 0–15 cm, and these sub samples were pooled. In the orange grove, seven rows were randomly selected and each row was considered a replication (plot). Three sub samples were collected from each row and at each 10 trees, and then pooled to form a sample. Composite samples were taken from every four rows, under the canopy of the trees. All samples were packed in plastic bags, stored in coolers, transported to the laboratory. Then they were sieved (2 mm) and divided into two parts, one for microbiological analysis, which was stored in a refrigerator at 4°C until further use, and another for chemical analysis, which was air-dried and kept at room temperature.

The media described by Bunt and Rovira (1955) and Martin (1950) was used to count total soil bacteria and total fungi, respectively. The soil samples were suspended in a solution of pyrophosphate 1% (w/v) and diluted up to 10^{-7}. Aliquots of these suspensions were added to culture media and incubated for 72 hours (bacteria) and 96 h (fungi) at a temperature of 30° C. The number of colonies was counted according to Vieira and Nahas (2005).

Respiratory activity was determined using 100 g soil (dry weight), as described by Rezende et al. (2004), with the moisture corrected to 60% of the water holding capacity (WHC). The soil samples were incubated in 2.5 liters sealed flasks containing two beakers with water and NaOH 0.5 M respectively. After two days of incubation at 30°C, $BaCl_2$ 30% (w/v)

was added to the beaker containing NaOH, and was then titrated with HCl 0.5 M up to pH 7.0 to determine the amount of CO_2 released. The potential nitrification was determined after soil incubation for 30 days with or without the addition of 160 µg NH_4-N ($(NH_4)_2SO_4$) g^{-1} dry soil and a moisture content of 60% WHC, and the N was determined by H_2SO_4 digestion and titration with 0.0025 M.

Urease activity was determined using 2.0 g soil by adding 1.0 mL of 10% urea as the substrate (McGarity and Myers, 1967). The amount of NH_4^+ released after incubation of soil samples for 3 hours was quantified by the indophenol method in a spectrophotometer at 630 nm. Urease activity was expressed in µg NH_4^+-N g^{-1} soil dried $3h^{-1}$. Phosphatase activity was determined in 0.2 g of wet soil, using p-nitrophenylphosphate 0.03 M as a substrate plus acetate buffer 0.1 M pH 5.4 (Rezende et al., 2004). After incubation of soil samples for 60 min, 1 ml $CaCl_2$ 0.5 M and 4 ml NaOH 4 M were added. The p-nitrophenol (pNP) released in the reaction was quantified by spectrophotometry at 405 nm. Phosphatase activity was expressed in µg pNP released per gram of soil per hour.

All results were converted on an oven-dried basis for analysis. The data were log (x +10) transformed and subjected to analysis of variance using the SAS program. Means were compared by Tukey's test at P<0.05of confidence level. Pearson's correlation (r) analysis was performed in order to explore the possible associations existing between different variables.

Table 1
Texture and chemical properties of the soil under orange grove, forest, and pasture

Variables	Top orange grove	Bottom orange grove	Forest	Pasture
pH	5	5	4.7	4.8
OM, g 100 g^{-1}	1.53b	2.54ab	2.66a	1.94ab
Moisture, g 100 g^{-1}	3.3b	5.3a	4.5ab	3.68ab
P resin, mg dm^{-3}	19	49	10	3
K^+, $mmol_c dm^{-3}$	1.6	3.8	2.3	1.4
Ca^{2+}, $mmol_c dm^{-3}$	15	16	12	6
Mg^{2+}, $mmol_c dm^{-3}$	7	10	8	4
H^++Al^{3+}, $mmol_c dm^{-3}$	18	28	16	18
BS, $mmol_c dm^{-3}$	23.6	29.8	15.1	11.4
CEC, $mmol_c dm^{-3}$	41.6	57.8	31.1	29.4
V, %	57	52	49	39
WHC, %	28.8	30.8	33.1	27.6
Clay, g kg^{-1}	70	120	80	70
Silt, g kg^{-1}	60	60	70	30
Sand, g kg^{-1}	870	820	850	900
Soil texture	Sandy	Sandy	Sandy	Sandy

OM, organic matter; CEC, cation exchange capacity; BS, base some; V, base degree saturation; WHC, water holding capacity; %, g/100g.Values with distinct letters in the columns are different according to Tukey test (p< 0.05).

Results

The highest levels of organic matter and moisture were observed in the forest and B/ORANGE soils, and the lowest levels were found in the T/orange soil (Table 1). Significant differences (Tukey, p<0.05) were found between the results found in the T/orange and B/orange soils (moisture) as well as the T/orange and the forest soils (organic matter) (Table 1), corroborating the influence of topography and vegetation cover on these attributes. The counts of bacteria and fungi ranged respectively from 3.94 to 9.32 x 10^6 CFU g^{-1} dry soils and 1.37 to 6.41 x 10^4 CFU g^{-1} dry soils (Figure 1).

The number of bacteria decreased as follows: forest> citrus> pasture, but the only significant difference (p<0.05) in the counts was found between the pasture and forest soil (Figure 1A). The number of fungi decreased as follows: citrus> forest> pasture, with significant differences between the counts found in the orange grove soil and the forest and pasture soils (Figure 1B).

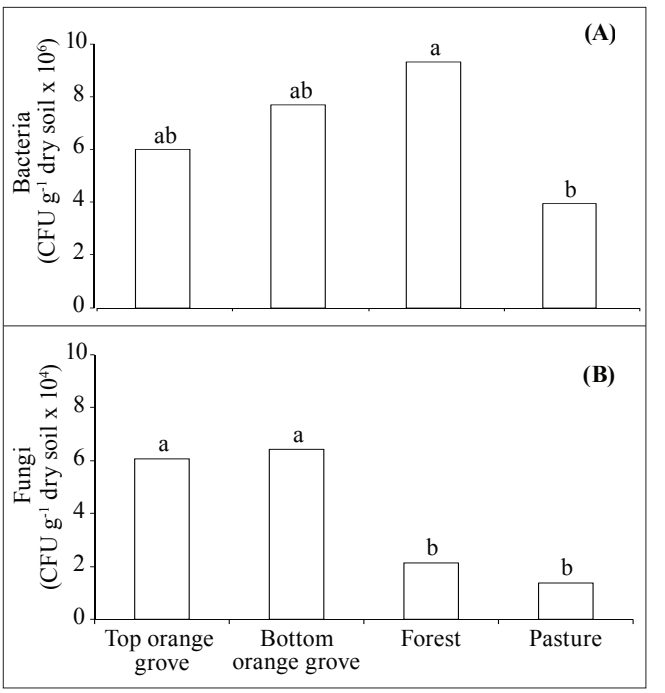

Fig. 1. Total bacteria (A) and fungi (B) of the soil under orange grove, forest, and pasture. Significant differences between means within columns based on Tukey test are indicated by different letters (p<0.05). CFU, colony-forming units

The highest respiratory activity was found in the forest soil, followed by B/orange, both of which differed significantly from pasture and T/orange soils (Figure 2A). The lowest respiratory activity was found in the pasture, producing about half the CO_2 observed in the forest soil.

The potential nitrification varied from 18.45 (pasture soil) to 25.60 mg N g^{-1} dry soil (forest soil) (Figure 2B). Although the difference was not significant, potential nitrification increased from 16 to 39% in the forest and orange soils, respectively, compared to pasture soil.

The same trend was observed in the activities of urease and phosphatase, i.e. these were higher in the forest, pasture, and B/orange soils in relation to T/orange soil (Figure 3A, B). However, the only significant difference was between the activities found in the forest and T/orange soils.

Simple correlation analyses (r) between individual soil properties are showed in Table 2. Respiratory activity showed a significant positive correlation with potential nitrification, phosphatase activity, and bacteria count. Significant positive correlations were also observed between urease activity and phosphatase activity as well as organic matter content. No correlation was found between fungi counts and the other soil variables.

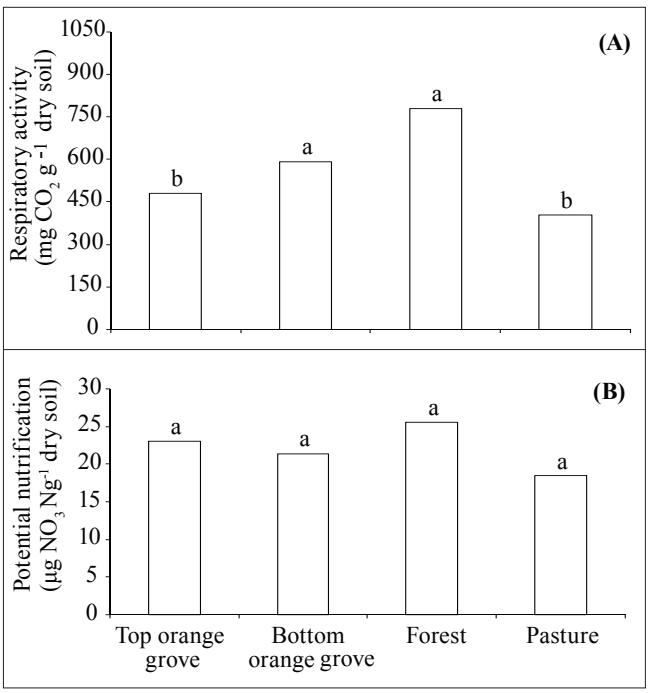

Fig. 2. Respiratory activity (A) and potential nitrification (B) of the soil under orange grove, forest, and pasture. Significant differences between means within columns based on Tukey test are indicated by different letters (p<0.05)

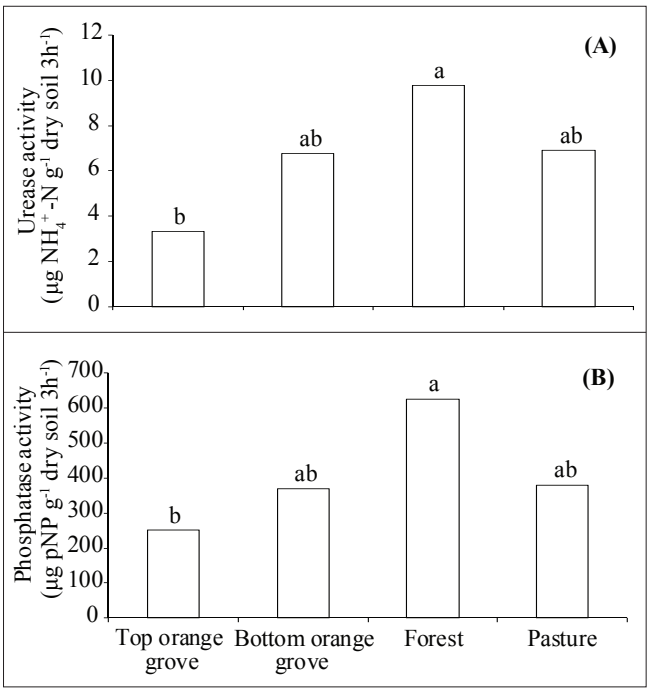

Fig. 3. Urease (A) and phosphatase activities (B) of the soil under orange grove, forest, and pasture. Significant differences between means within columns based on Tukey test are indicated by different letters (p<0.05). pNP, p-nitrophenol

Table 2
Pearson's correlation coefficients among soil attributes from soil under orange grove, forest and pasture

Variables	OM	Urease	Pot. nitrif.	Phosphatase	RA	Bacteria	Fungi
Moisture	0.583**	0.196^{NS}	0.106^{NS}	0.297^{NS}	0.239^{NS}	0.400*	0.084^{NS}
OM	-	0.426*	0.216^{NS}	0.378*	0.298^{NS}	0.463*	-0.049^{NS}
Urease	-	-	-0.159^{NS}	0.376*	0.324^{NS}	0.179^{NS}	-0.307^{NS}
Pot. nitrif.	-	-	-	-0.002^{NS}	0.444*	0.244^{NS}	0.083^{NS}
Phosphatase	-	-	-	-	0.512**	0.340^{NS}	-0.146^{NS}
RA	-	-	-	-	-	0.526**	0.095^{NS}

*p<0,05; ** p<0,01; NS= not significant; OM = organic matter; Pot. nitrif. = potential nitrification; RA = respiratory activity; N=28.

Discussion

Some research has shown that the number of bacteria in soils increase when forest or pasture were converted into agricultural areas. For example, the total number of bacteria was higher in maize crop soil than in pasture and forest soils (Pinto and Nahas, 2002). Waldrop et al. (2000) reported increased numbers of bacteria, fungi, and actinomycetes when forest was converted to pineapple crop. However, our data agree with those reported by Hai et al. (2004), who found a decrease in the numbers of microorganisms from forest to citrus and pasture soils. This response was evident for bacteria but not for fungi. The number of fungi decreased in the forest and pasture soils in relation to orange grove soils. A previous study reported that a number of environmental factors like pH, moisture content, and soil organic matter influence soil microbial populations (Kennedy et al., 2005). This response was evident for bacteria that increased with organic matter and soil moisture contents (Table 1), as indicated by the significant correlation coefficients of 0.463* and 0.400* respectively (Table 2). Fungi counts enhanced as soil phosphate content increased in the orange grove soils.

There is conflicting evidence regarding the effect of vegetation on respiratory activity. Arunachalam et al. (1999) reported that the production of CO_2 in the natural forest was twice that found in pasture soil. This is in agreement with our results. Schipper and Sparling (2000) reported increased respiratory activity in pasture and forest soils compared with cultivated soil. In contrast to these findings, respiratory activity of the B/orange soil was higher than that of pasture soil. A significant correlation was found between the production of CO_2 and the bacterial count (r = 0.526 **) but not the fungal count (r = 0.095 NS). Thus, variation in the nutrient concentrations in forest, pasture, and orange grove soils (Table 1) may have influenced bacterial growth and led to a decrease in respiratory activity.

In contrast to the findings of Ralte et al. (2005), no significant differences were found in the potential nitrification between the soils in our study. Factors such as NH_4^+, pH and soil moisture have been reported to influence the activity of nitrifying bacteria (Krave et al., 2002). However, none of these factors influenced potential nitrification of the soils studied, although the orange crop was fertilized with ammonium nitrate and soil moisture was significantly higher in the forest and B/orange soils in relation to others soils. Also no significant effect of the content of organic matter on the potential nitrification was observed (Table 2).

The preponderance of urease and phosphatase activities in forest soil in relation to other soils, and in B/orange compared to T/orange, may be related to the higher bacterial counts, respiratory activity and organic matter content of these soils. However, organic matter was only significantly correlated with urease (r = 0.426*) and phosphatase activities (r = 0.378*). Our data are consistent with previous reports, which found that the activity of several soil enzymes was related to the organic carbon content (Saviozzi et al., 2001). In addition, the significant correlation between bacterial counts and organic matter content (r = 0.463*) and respiratory activity (r = 0.526*) may suggest a possible influence of the growth of soil bacteria on the enzymes studied.

Carpenter-Boggs et al. (2003) observed that the acid phosphatase activity in pasture soil was almost triple that seen in no tillage and conventional tillage soils, with no significant difference between the latter. In this study, acid phosphatase activity in the forest soil was approximately twice that observed in the pasture and orange grove soils. Staley et al. (2008) found higher acid phosphatase activity in forest compared to pasture. The lower acid phosphatase activity in the orange grove soil may be attributed to a repressive effect due to the higher concentration of resin P in the soil (Table 1) (Rezende et al., 2004). The phosphatase activity in B/orange soil was 47% higher than that in T/orange soil. This higher phosphatase activity may be related to the increase in respiratory activity in the B/orange soil (Carpenter-Boggs et al., 2003).

Although the soil had a low water holding capacity, the stocking rate of the pasture (1–2 animals ha^{-1}) was comparable to the national average, and orange production in the 2004/2005 crop season (three boxes of 40.8 kg $plant^{-1}$) was higher than the Sao Paulo State average (2.5 boxes $plant^{-1}$). While the nutrient concentrations, microbial counts, and microbial activities were lower in the pasture soil than observed in the forest soil, these characteristics, with the exception of the fungal counts, were similar between forest and B/orange soil. Some considerations can be made. The stocking rate was achieved due to a programme of cattle rotation and the occasional addition of lime. The animal excreta was not enough to improve the soil quality of the pasture with the nutrient input resulting from the mineralization of organic matter, as expected (Carran and Theobald, 2000). The results found in the orange grove soil probably reflect the periodic fertilization. Another observation that should be highlighted is that sweeter and larger oranges (in both weight and diameter) were harvested from B/orange compared to T/orange.

The high accumulation of nutrients in the B/orange soil may have contributed to these results. Therefore, microorganism growth and consequently microbial activity in the B/orange soil may have been stimulated, which enhanced organic matter mineralization. Consequently, more nutrients were

found in the B/orange soil compared to the T/orange soil. For example, the phosphatase and urease activities were 1.5 and 2 times greater, respectively, in the B/orange soil than the T/orange soil. This increase in enzyme activity may have been due to the higher amount of organic matter in the B/orange soil compared to the T/orange soil. Chaer et al. (2009), who found close relationship between organic carbon content and the enzymes phosphatase, β-glucosidase, laccase, N-acetyl-glucosaminidase, protease, and urease, previously reported this effect. In addition, greater activity of enzymes such as dehydrogenase, urease, and phosphatase was observed in soil on a north-facing slope than on a south-facing slope in the northern hemisphere, due to variation in nutrient availability (Kang et al., 2009). Moreover, a possible significant influence of soil moisture content found in the T/orange soil (Table 1) in the activity of these enzymes may be suggested. The increase in urease activity can also be attributed to the considerable increase in the clay content found in B/orange soil (120 g kg^{-1}) in relation to the T/orange soil (70 g kg^{-1}) due to their mobilization on the slope. Sidari et al. (2008), who reported that topographical differences could influence microbial activity and organic matter content, supported this.

Conclusions

In conclusion, this study showed that the microbial properties assessed might be considered sensitive measures of changes in the ecosystems studied. Soil microbiological (bacteria and fungi counts) and biochemical properties (respiratory activity, potential nitrification, and the urease and phosphatase activities) related to the biocycles of the elements (C, N, and P) were used as indicators of soil quality. These results demonstrate that conversion of forest into pasture and then into orange crop results in soil changes indicative of decreased soil quality. Forest is recognizing as a sustainable ecological system and was used as reference having the best microbiological (except for fungi counts) and biochemical properties. The conversion of forest into pasture and the T/orange has damaged the soil, with decreased values of the properties studied. The pasture soil showed the worst quality and orange soil intermediate quality. However, in general no significant differences were found between the values in the forest and bottom of the orange grove soils. The results found in the B/orange soil can be attributed to the vegetation, the slope, and the chemical properties studied. The fertilization regime may also have contributed to our results, by stimulating growth and microbial activity.

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